

THE CHROMOSOMES OF PSEUDOCOCCUS NIPÆ.

FRANZ SCHRADER,

BRYN MAWR COLLEGE.

INTRODUCTION.

In the course of some work on sex determination in the different species of *Pseudococcus*—a genus of the Homoptera—very peculiar conditions were met with in the chromosome behavior. These peculiarities were observed especially in *Pseudococcus nipæ* (identified by H. Morrison, Bureau of Entomology), and it is of this species that the present account is given. A more detailed report, covering other species of *Pseudococcus* as well, is reserved for a later paper.

Most of the material was fixed in Allen's modification of Bouin's fluid. On the whole, fixation is more or less difficult; and at best the cells are somewhat small. The main features are clear cut, however, and hardly to be mistaken. I am indebted to Professor E. B. Wilson and to Professor C. E. McClung who examined some of my slides and offered helpful criticism.

THE CHROMOSOMES IN THE FEMALE.

The number of somatic chromosomes in the female is ten, with little or no size and form differentiation. Counts are made with little difficulty in various cells, but oögonial cells furnish of course the best criterion. Generally the chromosomes are counted most easily just before they have become arranged in a metaphase plate. There can be no doubt as to their number (Fig. 6). A detailed study of the maturation phenomena in the female was not made. Suffice it to say that five tetrads are formed and that these are normal in appearance; they are very much like those formed in the oögenesis of many other Homoptera (Fig. 7). The reduction process is thus probably not unusual.

SPERMATOGENESIS.

The somatic number of chromosomes in the male is also ten, and as in the female these seem to be alike in size and shape.

Such chromosome counts were generally made in cells of the developing nervous tissue where division phases are common (Figs. 3 and 4).

Spermatogonial divisions seem to be completed with comparative speed, for specimens which show them are not plentiful. Just as in the somatic cells the number here is undoubtedly ten (Fig. 5).

The stage following the spermatogonia seems to be much longer in duration. The cells increase perceptibly in size during this time. The earliest phase observable shows some flocculent masses of lightly staining chromatin irregularly distributed through the nucleus. At one point, always at the periphery of the nucleus, there is a more deeply staining mass. Nothing concerning the structure of this can be made out and its shape is variable (Fig. 8). With progressive development this deeply staining mass undergoes a few, very definite, changes. In successive steps it appears that a number of more or less irregular lumps is evolved. Still massed at first, these gradually become separated and then it is certain that they are five in number (Figs. 8 to 11). It is at this latter stage that a split is occasionally visible in some of them, but with increasing condensation this again becomes obliterated. Throughout this development these five bodies retain a definite tendency to remain in close proximity to each other, and this tendency is one that persists also through subsequent stages.

In the meanwhile the flocculent and more lightly staining chromatin has also undergone development. Before the denser mass has become evolved into five distinct bodies, this chromatin has been transformed into a fine network of threads. Apparently these are polarized toward the dense mass (Fig. 9). Like the leptotene threads of other forms, these threads shorten and thicken, a process accompanied by a progressive increase of their staining intensity. Polarization is finally lost, and already at this stage it becomes apparent that the number of shortened threads is less than ten (Fig. 10). As the threads continue their process of shortening, they are counted with greater ease, and in such a stage as shown in Fig. 11 it becomes certain that they are five in number. Like the denser bodies, these sometimes show a longitudinal split.

Both the denser bodies as well as the threads continue progressive condensation, and the former reach their final form some time ahead of the latter (Fig. 12). They are then somewhat oblong in shape, and take the hæmatoxylin stain with great intensity and there can be no doubt of their chromosomal nature. Somewhat later the erstwhile threads have also assumed this form, and there are then ten of these bodies or chromosomes, identical in size and shape. Those first evolved continue to betray a certain affinity for each other, and in the metaphase plate constitute a central group around which the other five chromosomes become ranged in no definite order (Figs. 13 and 14). Aside from this very characteristic grouping, the only difference between the two sets of chromosomes that is apparent consists in the rate at which they evolve or the stage which is the starting point of their development.

Throughout this development, there has been no trace of a tetrad formation. The general features of the case indicate that the split which was spoken of as occurring at one stage is nothing more than preparation for the equational division or else something of the nature of the "Querkerbe" observable in lower Crustacea.

Division now occurs in ordinary manner and ten chromosomes go to each pole (Figs. 15 to 17). The arrangement of chromosomes in the daughter cells is not absolutely certain, although fig. 16 indicates that there also the characteristic grouping is retained. Figures like these are too rare to admit of any definite conclusion however. At any rate, the time in which such an arrangement persists must be very short, for the chromosomes are generally found in a more or less irregular heap (Fig. 18).

The division just described is undoubtedly equational in character. Following it there seems to be no intervening further development in the chromosomes of the daughter cells. Instead, they begin to scatter in a longitudinal direction. This process is not entirely irregular however for it results in their separation into two groups of five each (Figs. 18 to 22). It is a remarkable feature that these two groups are each characterized by a distinct and different arrangement of their component chromosomes. The group going to one pole assumes the form of a V or a

triangle, while the sister group which goes to the opposite pole is circular or lumped in arrangement (Figs. 21 and 22). This grouping is so constant and has been observed in so many specimens, that no mistake seems possible, and the conclusion seems inescapable that it is of some significance. The telophase of this anomalous division still shows traces of the arrangement, but these are soon lost as the chromosomes of each daughter cell distribute themselves around the periphery of the nucleus. Their number here is undoubtedly five (Fig. 24). This initiates the formation of the spermatids in which the chromosomes gradually lose their staining reaction. No study of the subsequent stages was made except to determine that there is no sign of degenerating or abortive cells nor a size dimorphism in the spermatozoa.

SOMATIC CHROMOSOMES.

Returning to the somatic tissues, it may be remarked here that although the number of chromosomes in each sex is the same, their arrangement differs in the two sexes. This is especially noticeable in the developing nerve tract, where in the male the cells in the resting stage show a relatively large nucleolus like structure (Fig. 2). This is not to be seen in the same tissue in the female where cells show only the flocculent chromatin peculiar to that phase (Fig. 1). That the nucleolus-like structure in the male nerve cells is nothing but the group of five chromosomes mentioned in the description of the spermatogenesis becomes almost certain in metaphase plates found in the same tissue. Figs. 3 and 4 show such grouping without a doubt.

Exactly the same feature is observable in spermatogonial plates, though the size of these renders them less favorable (Fig. 5). In contradistinction, oogonial plates have no such arrangement, and even in such a late stage before division as shown in Fig. 6 the chromosomes are arranged in no definite order.

DISCUSSION.

An interpretation of these observations is perhaps not out of place. It is here given with the idea that it should not affect the observations however it may be received and is advanced in a speculative way.

As has already been stated, oögenesis very probably follows ordinary lines. The ten chromosomes constituting the diploid number are composed of five homologous pairs, and these synapse and form tetrads. Reduction is very probably normal, and results in a pronucleus with five chromosomes.

In the spermatogenesis, the spermatogonial divisions, like the somatic divisions, also occur in orthodox manner. This is apparently not true of the meiotic divisions however. In explanation of these, the best hypothesis is one which views the various developments in the light of sex chromosomal behavior and is as follows:

The central group of chromosomes which appears in the growth stages of the male as the more densely staining mass contains sex chromatin, equally distributed among the five chromosomes. The remaining chromosomes, which stain lightly at first, represent what may be regarded as purely autosomal chromatin. Granting this, and the assumption does not appear unjust in the light of what has been described, the seemingly peculiar development becomes a natural consequence. Just as in the spermatogenesis of the various Orthoptera and Hemiptera, the sex chromosomes always stain more or less intensely, and as far as observable do not go through the various stages of thread formation. That such formation may occur earlier, or in a restricted sense even while the dense mass is still irregular in outline, is not ruled out by any means. The autosomal chromatin on the other hand goes through all the usual steps, culminating in the formation of five chromosomes. The sex chromatin contained in the five grouped chromosomes will tend to explain their grouped arrangement, since again as in the Hemiptera, multiple X or X and Y chromosomes show a tendency to remain in close proximity during development.

If now the sex chromosomes in *Pseudococcus nipæ* are regarded homologous in every way to the autosomes, except that each carries a certain amount of sex chromatin, the subsequent behavior is just as would be expected. I may mention here that such sex chromosomes would imply a more intimate union of sex and autosomal chromatin than is illustrated by such a case as

Mermiria (McClung, '05) where the sex chromosome and the autosomes are distinct, but the former is attached to one of the latter. It is at present unnecessary to go into the relation of the two conditions, though very possibly they represent two distinct steps in the phylogeny of sex chromosomes.

Their subsequent behavior is more or less analogous to that of the X Y pair in other forms. This pair does not form a tetrad in the ordinary sense simply because its members are not homologous, or better perhaps, because neither has a true synaptic mate. When as in the homozygous state both members of a pair of sex chromosomes are homologous, synapsis and tetrad formation occur just as in the autosomes. This fact is plainly borne out in the oögenesis of many Hemiptera (Morrill, '10) as well as in the growth and maturation phenomena of the eggs of *Pseudococcus nipæ*. It is thus to be assumed that if in the present case of the spermatogenesis of *Pseudococcus nipæ* the sex chromatin were distributed equally over the ten autosomes, the pairs would be homologous and tetrads would be formed in the usual way.

The cytological evidence indicates nothing that should render an equational division exceptional in nature, and it does indeed occur in the usual manner. The second division witnesses reduction in that the autosomes carrying sex chromatin go to one pole while the purely autosomal chromosomes go to the opposite pole. Taking recourse to a parallel case once more, attention may be drawn to the two X chromosomes in *Syromastes* which always go the same pole in reduction (Wilson, '09). Similarly, the multiple X of the Reduviidæ always goes to one pole, although this is not an exactly parallel case since it is probably the product of fragmentation of a single X.

Thus to repeat what has already been intimated for the present case, the distribution of the chromosomes to their respective poles in the reduction division may be explained on the ground that we are concerned with five pairs of chromosomes. The members of each pair are homologous except for the fact that one of them in each instance carries a certain amount of sex chromatin. The presence of the latter does not influence the behavior of the chromosome pairs in reduction and the members of each pair go

to opposite poles. Its presence does however prevent haphazard distribution in that the five chromosomes carrying this sex chromatin tend to remain clustered or grouped and therefore go to the same pole.

Although more or less contrary to the cytological evidence furnished by other groups of insects, it may not be amiss to suggest the possibility that in animals with haploid males each chromosome carries a certain amount of sex chromatin. It follows that the diploid female would then represent $2 X$, whereas the haploid male would represent $1 X$. In the haploid male the reduction division is not truly abortional as has been supposed, but is merely a division in which these sex chromatin carrying chromosomes go to one pole while the opposite pole receives no chromosomes simply because the mates to these chromosomes are absent. It is of interest to note that the straggling or lagging so often observed in the sex chromosomes of various insects is paralleled by the scattered and irregular distribution of the chromosomes on the spindle of this division in the Hymenoptera. And lastly, such irregular distribution is found also in the reduction division of *Pseudococcus nipæ*.

Pseudococcus nipæ thus would stand half way between forms with haploid males where every chromosome carries sex chromatin, and forms in which the sex chromatin is carried in very few chromosomes and there is little numerical variation in the chromosomes of the two sexes. In other words, half of the chromosomes in the males carry sex chromatin.

Although superficially an instance of Weismann's postulated ideal type of reduction in which the diploid number of chromosomes is halved without previous syndesis, the spermatogenesis of *Pseudococcus nipæ* nevertheless follows the commonly accepted lines of meiosis. The apparently exceptional behavior can be explained as due to an extreme mode of sex chromatin distribution and is not a unique example of the *Primartypus* of reduction. It may be remembered that Goldschmidt ('05 and '08) gave this name to an instance of Weismann's simple type which he thought to have discovered in *Zoögonus mirus*. The Schreiners ('08) examining Goldschmidt's slides believed to have

found a serious error in his counts of somatic chromosomes, which they believed in reality to be 24 and not 10 as he had reported. Furthermore, reduction occurred in the ordinary way, just as in *Tomopteris*. Gregoire ('09) in going over the same slides maintained that the Schreiners were correct in that the case was one of ordinary reduction, but that they in turn had made an error in the chromosome counts. The somatic number is about 12, and the reduced number 6. Lastly Wassermann ('11, '12, and '13) procured new material and concluded that Gregoire's counts had been correct. He did not agree with Gregoire as to the mode of synapsis however, and apparently was unable to reach a final conclusion in this regard himself. Although he thus does not believe that the question has received a definite settlement, the fact remains that *Zoögonus* does not represent the simple type of reduction that Weismann advanced in a hypothetical way.

If my hypothesis is correct, the male of *Pseudococcus nipæ* is heterozygous in that it has five sex-chromatin carrying chromosomes and five chromosomes purely autosomal in character. Crossing over would not occur in these chromosomes. It would occur however in the female, in which the ten chromosomes are composed of five homologous pairs. If the male represents 1 X, the female with ten sex-chromatin carrying chromosomes represents 2 X.

SUMMARY.

1. The diploid number of chromosomes in *Pseudococcus nipæ* is ten in both sexes.

2. In the maturation of the egg, five tetrads are formed and reduction is probably normal.

3. In the spermatogenesis, five chromosomes are developed before the others, and these tend to remain grouped together.

4. No tetrads are formed, and in reduction five chromosomes go to one pole (supposedly those evolved first) and five to the other.

5. Explanation of this seemingly anomalous behavior is to be sought in the fact that five of the chromosomes carry sex chromatin.

6. The case is not so much to be regarded as an illustration of Weismann's ideal type of reduction, as an exceptional example of reduction due to unusual sex chromatin distribution.

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All drawings made with a Spencer 15 eyepiece and Zeiss 1.5 objective with the exception of Figs. 1 and 2, where a 10 eyepiece and 2 mm. objective were used.

PLATE I.

1. Cells in nervous tissue of the female.
2. Cells in nervous tissue of the male.
- 3 and 4. Metaphase plates from nerve tissue of the male.
5. Spermatogonial plates.
6. Oögonial cell.
7. Tetrads prior to polar body formation in the egg.
- 8 to 12. Growth stages prior to first division.
13. Metaphase plate of first division.

